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Evaluation of in-vitro antimicrobial activity of 2-aryloxy methyl oxazoline analogues

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ABSTRACT

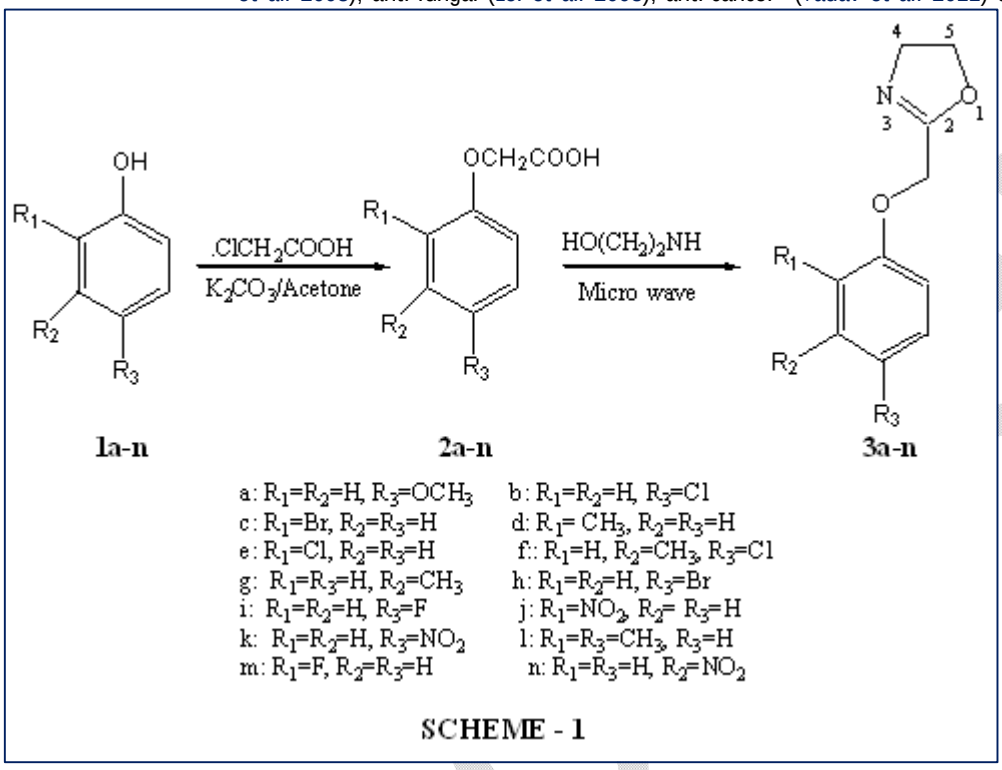
There has been a dramatic increase in pathogen resistance to both pharmaceutical and agrochemical antimicrobial agents. New prototypes (lead compounds) are needed to address this situation. In this connection, oxazoline analogues **3a-were** evaluated in vitro for their efficacy as antimicrobial agents against representative strains of Gram-positive (*S. aureus, S. aureus (MRSA), E. aerogens, M. luteus.*) and Gram-negative bacteria (*K. pneumonia, S. typhimurium, S. paratyphi- B, P. vulgaris*) and, fungi (*C. albicans, B,. cinerea, M. Pachydermatis, C krusei*) by disc diffusion and minimum inhibitory concentration studies. Among the series **3a-n**, compounds **3b, 3c, 3e, 3h**, **3i** and **3m**showed potent antimicrobial activities,when compared to the standard drug.

Keywords: In-vitro antimicrobial activity, oxazoline.

1. INTRODUCTION

Antibiotics are one of our most important weapons in fighting bacterial infections and have greatly benefited the health-related quality of human life since their introduction (Appelbaum & Hunter,2000; Ball, 2000). Howeverin the current scenario, there is an alarming increase in life threatening microbial infections especially in immune compromised individuals suffering from cancer, AIDS, etc (Vanden et al. 1998; Andriole, 1998; Boruah & Skibo, 1994; Ghannooun & Rice, 1999). There has been a constant effort by the scientists to develop more effective and safe antimicrobial drugs to battle with microbial infections (Graybill, 1992; Rinaldi, 1992; Walsh, 1993; Warnock, 1995;

Georgopapadakou & Walsh, 1996). In spite of the development of several new antimicrobial agents, their clinical value is limited to treat an increasing array of life threatening systemic infections because of their relatively high risk of toxicity, emergence of drug resistant strains, pharmacokinetic differences, and/or inadequacies in their antimicrobial activity (Emami et al. 2004). Therefore, a great need for a more potent and broad spectrum antimicrobial agents with reduced side effects (Graybill, 1996). Also, it is essential to investigate newer drugs with lesser resistance. Systematic studies among various pharmacological compounds have revealed that any drug may have the possibility of possessing diverse functions and thus may have useful activity in completely different spheres of medicine.For instance, nitrogen heterocycles in general oxazoline and its analogues in particular are the most exploring fields in heterocyclic chemistry due to their diverse biological activities such as anti-inflammatory (Khanum et al. 2008), anti-fungal (Lei et al. 2008), anti-cancer (Yadav et al. 2011) and anti-bacterial (Correia et al. 2011) activity. In continuation of our(Khanum et al. 2005)ongoing program to develop antimicrobial agents has inspired us to evaluate in vitro antimicrobial study of 2-aryloxy methyl oxazoline analogues.



SCHEME - 1

2. EXPERIMENTAL

The synthetic sequence is outlined in Scheme 1. A mixture of **1a-n**, chloro acetic acid in acetone and anhydrous potassium carbonate was refluxed for 8 h, cooled and the solvent removed under reduced pressure. The residual mass was triturated with ice water to remove potassium carbonate, extracted with ether and the ether layer was washed with 10% sodium hydroxide solution followed by distilled water. The ether layer was dried over anhydrous sodium sulphate and evaporated to dryness to get crude solid, which on recrystallization with ethanol gave pure substituted aryloxy ethanoic acids (**2a-n**). A mixture of **2a-n** and ethanolamine was subjected to microwave irradiation operating at its 20% power for 5-10 min. The reaction mixture was extracted into ether, washed with distilled water and dried over anhydrous sodium sulphate. After evaporation of ether layer the crude solid was recrystallized with ethanol to afford 2-aryloxy methyl oxazolines **(3a-n**). The compounds **2a-n**and **3a-n**were characterized by IR, ^{1}H NMR and mass spectrophotometer (Khanum et al. 2008).

3. PHARMACOLOGY

3.1. Materials and methods for the antimicrobial activity

Streptomycin and ciprofloxacin (Sigma) were used as positive controls against bacteria. Fluconazole and ketoconazole (Himedia, Mumbai) were used as positive controls against fungi.

3.2. Tested microbes

The following gram positive bacteria were used for the experiments; *S. aureus, S. aureus (MRSA), E. aerogens, M. luteus*. The gram negative bacteria included, *K. pneumonia, S. typhimurium, S. paratyphi- B, P. vulgaris*. In addition, fungi *C. albicans, B,. cinerea, M. pachydermatis, C krusei* were also used for the experiments. All cultures were obtained from the Department of Microbiology, Manasagangotri, Mysore.

3.3. Preparation of inoculums

Bacterial inoculums were prepared by growing cells in Mueller Hinton Broth (MHA) (Himedia) for 24 h at 37 ^{o}C. These cell suspensions were diluted with sterile MHB to provide initial cell counts of about 104 CFU/ml. The filamentous fungi were grown on sabouraud dextrose agar (SDA) slants at 28 ^{o}C for 10 days and the spores were collected using

Table 1
In-vitro antibacterial activity of compounds **3a-n**

Compounds	Zone of inhibition in mm							
	Gram positive bacteria		Gram negative bacteria					
	S. aureus	*S. aureus (MRSA)*	*E. aerogens*	*M. luteus*	*K. pneumonia*	*S. typhimurium*	*S. Paratyphi-B*	*P. vulgaris*
3a	10	9	9	11	9	11	8	12
3b	25	17	18	21	16	23	16	19
3c	24	14	19	17	15	24	9	18
3d	12	10	13	11	9	15	9	11
3e	23	20	25	27	27	29	30	29
3f	10	11	12	11	12	9	8	9
3g	11	11	10	11	9	10	8	9
3h	21	15	20	21	15	24	13	17
3i	20	12	14	16	17	19	12	13
3j	11	8	9	12	11	10	8	9
3k	16	12	15	13	14	16	9	10
3l	10	9	9	10	9	11	8	10
3m	19	12	14	15	16	18	11	18
3n	17	13	16	14	15	17	10	11
Streptomycin	18	21	23	26	23	25	19	25

sterile doubled distilled water and homogenized. Yeast was grown on sabouraud dextrose broth (SDB) at 28 ^{o}C for 48 h.

3.4. Disc diffusion assay

Antibacterial activity was carried out using a disc diffusion method (Murray et al. 1995). Petri plates were prepared with 20 ml of sterile Mueller Hinton Agar (MHA) (Himedia, Mumbai). The test cultures were swabbed on the top of the solidified media and allowed to dry for 10 mins. The tests were conducted at 1000 mg/disc. The loaded discs were placed on the surface of the medium and left for 30 min at room temperature for compound diffusion. Negative control was prepared using respective solvent. Streptomycin (10 mg/disc) was used as positive control. The plates were incubated for 24 h at 37 °C for bacteria and 48 h at 27 °C for fungi. Zone of inhibition was recorded in millimeters and the experiment was repeated twice.

3.5. Minimum inhibitory concentration (MIC)

Minimum inhibitory concentration studies of compounds were performed according to the standard reference method for bacteria (Duraipandiyan & Ignacimuthu, 2009) and filamentous fungi (Rex et al. 2008).Required concentrations (1000 mg/ml, 500 mg/ml, 250 mg/ml, 125 mg/ml, 62.5 mg/ml, 31.25 mg/ml and 15.62 mg/ml) of the compound was dissolved in DMSO (2%), and diluted to give serial two-fold dilutions that were added to each medium in 96 well plates. An inoculum of 100 ml from each well was inoculated. The anti-fungal agents ketoconazole, fluconazole for fungi and streptomycin, ciprofloxacin for bacteria were included in the assays as positive controls. For fungi, the plates were incubated for 48-72 h at 28°C and for bacteria the plates were incubated for 24 h at 37 °C. The MIC for fungi was defined as the lowest extract concentration, showing no visible fungal growth after incubation time. 5 ml of tested broth was placed on the sterile MHA plates for bacteria and incubated at respective temperatures. The MIC for bacteria was determined as the lowest concentration of the compound inhibiting the visual growth of the test cultures on the agar plate.

4. RESULTS AND DISCUSSION

Compounds **3a-n**has been prepared as previously reported by our group (Khanum et al. 2008). The antimicrobial activities of compounds **3a-n**were screened against eight bacteria and four fungi using in-vitro disc diffusion method. The results revealed that most of the compounds exhibited antimicrobial activities against *Staphylococcus aureus, Staphylococcus aureus (MRSA), Enterobacter aerogens, Micrococcus luteus, Klebsiella pneumonia, Salmonella typhimurium, Salmonella paratyphi-B, Proteus vulgaris, Candida albicans, Botyritis cinerea, Malassesia pachydermatis,* and *Candida krusei*organisms. The results are summarized in Table 1 and 2. Compounds **3b, 3c, 3e, 3h**, **3i**and **3m**

Table 2

In-vitro antifungal activity of compounds **3a-n**

Compounds	Zone of inhibition in mm			
	C. albicans	*B. cinerea*	*M. pachydermatis*	*C. krusei*
3a	9	8	10	11
3b	14	10	26	16
3c	14	10	25	13
3d	10	9	15	14
3e	18	16	21	23
3f	9	8	10	7
3g	10	9	9	11
3h	13	14	11	19
3i	12	10	13	20
3j	9	13	8	8
3k	9	12	13	15
3l	11	13	15	14
3m	23	9	12	15
3n	11	9	13	10
Ketoconazole	22	14	24	18

Table 3

MIC (mg/ml) of compounds tested against bacteria

Compounds	Minimum inhibitory concentration (□g/ ml)							
	Gram positive bacteria			Gram negative bacteria				
	S. aureus	*S. aureus (MRSA)*	*E. aerogens*	*M. luteus*	*K. pneumonia*	*S. typhimurium*	*S. Paratyphi-B*	*P. vulgaris*
3b	15.62	250	250	32.5	250	31.25	500	62.5
3c	15.62	125	62.5	125	250	15.62	500	125
3e	31.25	62.5	15.62	15.62	15.62	15.62	125	<15.62
3h	15.62	250	62.5	62.5	550	31.25	500	125
3i	31.25	125	125	63.5	63.5	250	125	250
3m	62.5	500	500	250	250	125	500	500
Streptomycin	6.25	>100	25	6.25	6.25	30	ni	6.25
Ciprofloxacin	<0.78	>100	<0.78	>100	<0.78	>100	6.25	<0.78

ni = no inhibition

showed good activity more than standard drug against S. aureus. Compound **3e** with a chloro group at another position in the phenyl ring showed good activity against both Gram-positive and Gram-negative bacteria among theseries of compounds**3a-n** compared with the standard drug streptomycin. On the other hand, compounds **3d, 3f, 3g** and **3l**withmethyl groupand **3j, 3k** and **3n** with nitro group showed less activity. Interestingly compounds **3c** and **3h** with bromo group at ortho and para position respectively, and compound **3i** with the Fluoro group at the para position in the phenyl ring shown good activity against *S. aureus*. Compound **3e** showed significant antifungal activity against *B. cinerea* and C. krusei. Compounds**3h** with bromo group at para position and **3i** with the fluoro group at the para position showed more activity against *the* compared to standard drug. Further, compound **3m** with the fluoro group at ortho position showed more activity against *C. albican* and compounds**3b**and **3c** with chloro and bromo groups at para and ortho position respectively, showed more activity against *M. pachydermatis* strain. In contrast, compounds **3a,3d, 3f, 3g, 3j-l** and **3n**exhibited less activity. The MIC values of active compounds **3b, 3c, 3e, 3h**, **3i** and **3m**against bacteria and fungi are given in Table 3 and 4. Significant MIC values were observed against Gram positive and Gram negative bacteria. Compounds **3b, 3c** and **3h** showed good activity against *S. aureus*. In comparison to

Table 4

MIC (mg/ml) of compounds tested against fungi

Compounds	Minimum inhibitory concentration (µg/ ml)			
	C. albicans	*B. cinerea*	*M. pachydermatis*	*C. krusei*
3b	500	500	250	250
3c	250	250	500	250
3e	31.5	125	250	31.5
3h	500	250	500	250
3i	250	250	125	125
3m	500	500	250	250
Fluconazole	>100	ni	12.5	12.5
Ketoconazole	25	25	15	15

ni = no inhibition.

compounds **3e** and **3i**, in compounds **3b, 3c** and **3h**the potency against *S. aureus*have been increased by one fold. Interestingly the presence of chloro group in **3b** and bromogroup in **3c** and **3h**at the phenyl ring increased the potency against *S. aureus* by two fold compared to fluoro group in **3m.** In comparison to compound **3m** in compound **3e** the potency is increased by two fold against bacteria S. paratyphi-B, three fold by *S. aureus* (MRSA) and *S. typhimurium*, four fold by M. *luteus, K.* and *pneumonia* and fivefold by *E. aerogens*. Besides, the potency of compound **3e** is increased by two fold against fungi *B. cinerea*, three fold by *C. krusei*and four fold by *C. albicans* compared to compound **3m**. In general, compound **3e**showed better activity than standard drugs for most of the tested bacteria and fungi.

5. CONCLUSION

Investigation of antibacterial and antifungal screening data revealed that title compounds **3a-n**are new promising antimicrobials. These novel compounds were evaluated for their activities against eight bacteria and four fungi. Compound **3e** with a chloro group at ortho position in phenyl ring was found to be more than 1.6 times active against *S. aureus* (MRSA) bacteria than streptomycin and ciprofloxacin. Moreover more than 6.4 times active against *M. luteus* and *S. typhimurium* bacteria than ciprofloxacin. Compound **3e**was also found to be more than 3.2 times active against *C. albicans* fungi than fluconazole. In contrast, compounds **3a, 3d, 3f, 3g, 3j-l** and **3n** exhibited lowest activity.These results showed that synthesized compound **3e**might be a potential antibacterial and anti-fungal agent.

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